

Testing for evolutionary trade-offs in a phylogenetic context: ecological diversification and evolution of locomotor performance in emydid turtles

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Abstract

The evolution of ecological trade-offs is an important component of ecological specialization and adaptive radiation. However, the pattern that would show that evolutionary trade-offs have occurred between traits among species has not been clearly defined. In this paper, we propose a phylogeny-based definition of an evolutionary trade-off, and apply it to an analysis of the evolution of trade-offs in locomotor performance in emydid turtles. We quantified aquatic and terrestrial speed and endurance for up to 16 species, including aquatic, semi-terrestrial and terrestrial emydids. Emydid phylogeny was reconstructed from morphological characters and nuclear and mitochondrial DNA sequences. Surprisingly, we find that there have been no trade-offs in aquatic and terrestrial speed among species. Instead, specialization to aquatic and terrestrial habitats seems to have involved trade-offs in speed and endurance. Given that trade-offs between speed and endurance may be widespread, they may underlie specialization to different habitats in many other groups.

Introduction

The evolution of ecological specialization is a central topic that unites ecology and evolutionary biology, and is thought to be a major driver of adaptive radiations (Pianka, 2000; Schluter, 2000; Futuyma, 2005). Ecological specialization is thought to occur because of a trade-off between the ability to exploit many resources or habitats and the ability to exploit fewer more effectively (e.g. MacArthur, 1972; Futuyma & Moreno, 1988; Pianka, 2000; but see Fry, 1996). The term 'trade-off' is usually understood to mean a necessary negative correlation between two aspects of performance or fitness (e.g. Futuyma & Moreno, 1988; Fry, 1996; Vanhooydonck *et al.*, 2001; Roff & Fairbairn, 2007), and researchers have applied the term broadly to include negative correlations between various traits among individuals, populations,

and species (e.g. Van Ballegooijen & Boerlijst, 2004; Yoshida *et al.*, 2004; Miner, 2005).

Many ecological and evolutionary studies have sought evidence of evolutionary trade-offs between pairs of traits among species (e.g. Badyaev, 2002; Arendt, 2003; Poulin & Mouillot, 2004). However, the standard approach of looking for evolutionary trade-offs in terms of any negative correlation between traits is potentially problematic when applied at this level. A critical idea in many previous discussions of evolutionary trade-offs (e.g. Futuyma & Moreno, 1988; Losos & Miles, 1994; Pianka, 2000; Schluter, 2000; Irschick, 2002) is that improving performance at one task requires decreasing performance at another (or improving fitness in one setting requires decreasing fitness in another). Although this pattern of increases and decreases in performance traits may be expected to generate a negative correlation between traits among species, other processes could also give rise to a negative correlation. For example, given two performance traits among a set of species, a negative correlation between them could arise if the two performance traits simply increased in different lineages, without any evolutionary decreases in either trait

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(i.e. trait A increases in clade 1 and trait B increases in clade 2). We would argue that this pattern does not represent an evolutionary trade-off as thought of by most evolutionary biologists, even though it would be considered a trade-off under the standard definition of a negative correlation between traits. Given this problem, we think that a more explicit definition of an evolutionary trade-off is needed.

We here define an 'evolutionary trade-off' as an evolutionary increase in one aspect of performance or fitness (relative to the ancestral state) is associated with an evolutionary decrease in a related aspect of performance or fitness, on the same branch of the phylogeny. In other words, improving performance at one task is associated with decreasing performance at another (or improving fitness in one setting is associated with decreasing fitness in another). Given this definition, the most direct method of testing for evolutionary trade-offs is to use ancestral character reconstruction to determine if increases and decreases in two aspects of performance or fitness have been correlated. Surprisingly, this approach has almost never been used in past comparative studies of evolutionary trade-offs (but see Moran, 2004).

In this study, we use a phylogenetic approach to test whether evolutionary shifts between aquatic and terrestrial habitats in emydid turtles are associated with evolutionary trade-offs in locomotor performance in each habitat. The two major axes along which animal species divide niche space during ecological diversification and adaptive radiation are generally thought to be habitat and diet (Schluter, 2000). Evolutionary changes in locomotor ability may be critical for changes in habitat use, because a motile organism that cannot move effectively in a given habitat cannot gather resources or reproduce there. Trade-offs in locomotor performance associated with transitions between aquatic and terrestrial habitats are thought to have been important throughout vertebrate evolution. For example, trade-offs between aquatic and terrestrial locomotion are thought to have been important in the origin of limbs in tetrapods (e.g. Shubin *et al.*, 1997). However, there are relatively few groups in which it is possible to study trade-offs in locomotor performance among closely related aquatic and terrestrial species, given that few clades contain both fully aquatic and fully terrestrial species.

Emydidae is a family of turtles with 12 genera and 40 currently recognized species (Stephens & Wiens, 2003). Most species have been the subjects of numerous descriptive ecological studies (reviewed in Ernst & Barbour, 1989; Ernst *et al.*, 1994). The family includes highly terrestrial species (e.g. Ornate Box Turtle, *Terrapene ornata*) that forage exclusively on land and almost never enter the water, highly aquatic species (e.g. map turtles, *Graptemys*) that rarely leave the water, and semi-terrestrial species (e.g. Wood Turtle, *Glyptemys insculpta*) that spend considerable time in both aquatic and terrestrial habitats (Ernst & Barbour, 1989; Ernst *et al.*, 1994).

Because closely related emydid species occur in such diverse habitats, they are an unusually well-suited group in which to test whether the evolution of trade-offs in locomotor performance is involved in evolutionary diversification and specialization in habitat use.

Intuitively, trade-offs between aquatic and terrestrial locomotor performance (e.g. aquatic speed and terrestrial speed) seem likely because aquatic and terrestrial environments have such different biomechanical requirements for locomotion (e.g. Gillis & Blob, 2001; Biewener, 2003). Air and water have very different viscosities, and turtles are neutrally buoyant in water but not on land (Zug, 1971; Biewener, 2003). In emydid turtles, different morphologies seem to be associated with aquatic vs. terrestrial habitat use. For example, aquatic species have a large webbed pes, whereas terrestrial species have a smaller unwebbed pes and frequent digital reduction (Zug, 1971). To date, there has been only one phylogenetic comparative analysis of aquatic and terrestrial speed in vertebrates, a study which examined 10 species of semi-aquatic salamanders (Gvozdik & van Damme, 2006). Surprisingly, that study found no evidence of a trade-off between habitats.

Specialization to different habitats in emydids might also involve trade-offs between speed and endurance, as suggested by behavioural differences between aquatic and terrestrial species (Ernst & Barbour, 1989; Ernst *et al.*, 1994). When active, aquatic emydid species spend most of their time foraging in water, where the weight of their shell is buoyed. Thus, they may not need as much endurance as more terrestrial species, which often have to carry the full weight of their shell. In addition, aquatic emydids spend much of their time basking near water, and are known to rapidly flee into the water and swim away when disturbed, whereas the most terrestrial emydids (*Terrapene*) escape predation by remaining immobile and closing their hinged shells. Based on the physiological constraints of muscle tissue (e.g. Rome *et al.*, 1988; Rome, 2002) interspecific trade-offs between speed and endurance are assumed to commonly occur, although only two previous comparative studies have demonstrated them (Huey *et al.*, 1984; Vanhooydonck *et al.*, 2001). No previous studies have investigated how trade-offs between speed and endurance might be related to specialization to different habitats.

In this study, we obtained data on locomotor performance for 16 ecologically diverse species of emydid turtles and reconstructed a phylogeny for these same species based on morphological and molecular data. We measured both aquatic and terrestrial speed and endurance for as many of these 16 species as possible. By combining the phylogeny and locomotor data, we tested for evolutionary trade-offs between aquatic speed and terrestrial speed and between speed and endurance. These trade-offs are expected based on the differing biomechanical requirements of aquatic vs. terrestrial locomotion and the physiology of skeletal muscle

respectively. Finally, we tested whether species differed significantly in speed and endurance in association with their habitat usage.

Materials and methods

Analysis of locomotor performance

A brief outline of our methods is given below. For a more detailed description of the methods see Appendix S1. We sampled 16 emydid species for locomotor and phylogenetic analyses (Table 1), and included species usually considered to be terrestrial, semi-terrestrial, and aquatic in reviews of turtle ecological literature (e.g. Ernst & Barbour, 1989; Ernst *et al.*, 1994). We also included representatives of all currently recognized emydid genera, with the exception of the monotypic *Actinemys* (taxonomy follows Stephens & Wiens, 2003). Finally, we sampled species more densely in parts of the tree where evolutionary transitions between habitats seem to have occurred, based on Stephens & Wiens (2003).

Many emydid species show pronounced sexual size dimorphism (Ernst *et al.*, 1994). In order to obtain comparable data across all species, we included only adult males. The number of suitable individuals available varied somewhat between species and was limited in many cases (Table 1, mean = 3.68 individuals per species). Many of the relevant emydid species are uncommon or even endangered in the wild, and obtaining large numbers of adult males for all species would be virtually impossible. However, the range of variation in performance variables (see below) among species was much greater than the range of variation seen among individuals of any one species.

Aquatic locomotion trials were performed in two large aquariums, one constructed of Plexiglas and one of glass (the smaller glass aquarium was used to measure individuals of two highly endangered species at the Bronx Zoo). In each aquarium, an observation area was set up where a mirror (either above or beneath the observation area) was stationed at a 45° angle. This allowed the movements of specimens to be observed in three dimensions using a single camera positioned at a distance from the observation area. Terrestrial trials were generally performed in a track (100 cm long and 30 cm wide) with white foam-board walls and a surface of packed earth. Turtles were placed at one end of the track and motivated to walk past an observation area near the middle of the track. Over this observation area was placed a camera mounted on a tripod.

During preliminary trials with each species, a variety of stimuli (e.g. shell tapping, arm waving, or food luring) were used to determine which produced the highest observed speed. The particular stimulus that elicited the maximum speed in each species was then used for future speed trials. A total of at least 10 speed trials were performed with each specimen. Endurance trials consisted of repeated speed trials, and were only performed when a specimen had been used in no previous trials for at least one week (i.e. specimens were seemingly well rested). Species were motivated to swim or crawl past the observation area repeatedly, so that the decrease in speed over time could be measured. Intervals between speed trials were limited to 2 s. Most species only responded sufficiently to the stimulus used in speed trials to allow endurance to be measured in either terrestrial or aquatic environments, but not both. Thus, both aquatic and terrestrial endurance data were only obtained for four

Table 1 Summary of data on aquatic and terrestrial speed and endurance.

Taxon	Habitat type	<i>n</i>	Aquatic speed (absolute/relative)	Aquatic endurance	Terrestrial speed (absolute/relative)	Terrestrial endurance
<i>Chrysemys picta marginata</i>	A	5	42.72/2.69	0.62	45.40/2.86	0.40
<i>Deirochelys reticularia</i>	A	2	34.37/2.48	1.00	16.61/1.20	–
<i>Graptemys nigrinoda</i>	A	4	37.74/4.59	0.45	31.08/3.18	–
<i>Graptemys ouachitensis</i>	A	3	40.92/4.40	0.50	18.60/2.00	–
<i>Graptemys versa</i>	A	4	45.47/5.32	0.54	20.68/2.51	–
<i>Malaclemys terrapin</i>	A	8	37.24/3.22	0.75	35.47/2.39	0.82
<i>Pseudemys concinna</i>	A	2	42.46/3.56	0.45	15.30/1.28	–
<i>Trachemys scripta</i>	A	6	42.77/3.76	0.60	–	–
<i>Clemmys guttata</i>	A	4	23.16/2.53	0.65	25.31/2.31	–
<i>Emydoidea blandingii</i>	S	4	29.65/1.56	0.69	19.12/1.01	0.97
<i>Emys orbicularis</i>	A	2	26.18/2.08	0.67	–	–
<i>Glyptemys insculpta</i>	S	5	24.16/1.35	0.90	25.15/1.37	0.63
<i>Glyptemys mühlenbergii</i>	S	2	16.76/1.46	0.88	–	–
<i>Terrapene carolina</i>	T	3	14.66/1.21	–	19.16/1.68	1.00
<i>Terrapene coahuila</i>	S	2	28.56/1.55	1.00	21.99/1.11	–
<i>Terrapene ornata</i>	T	3	10.71/0.94	–	21.05/1.75	–

Habitat type refers to coding of species as aquatic (A), semi-terrestrial (S) and terrestrial (T) for ANOVA and ancestral trait reconstruction. Units of absolute speed are cm s⁻¹, units of relative speed are carapace lengths s⁻¹ and units of endurance are percentage of maximum speed observed after 20 min of continuous locomotion.

species. Three species in the study did not respond to any stimulus in terrestrial trials (showing no interest in food and retreating into their shells when exposed to any negative stimulus). Only aquatic speed data were obtained for these three species. We acknowledge that the behavioural motivation of specimens may have some impact on our results, and that our performance data may reflect a combination of physiological ability and behaviour (Irschick *et al.*, 2005).

All performance trials were recorded using a digital video recorder (Sharp DL-WD450 Digital Viewcam; Sharp Electronics Corporation, Mahwah, New Jersey, USA). Capture of videos and video editing were performed using ADOBE PREMIERE PRO (version 6.0.1 for Macintosh and version 7.0 for Windows; Adobe Systems, Inc., San Jose, California, USA). Digital image analysis was performed using OPTIMAS version 6.5 (Optimas Corporation, Fort Collins, Colorado, USA) in the Functional Ecology Research and Training Laboratory in the Department of Ecology and Evolution at Stony Brook University. Given that species differ considerably in body size, we used relative speed (carapace length per second) as our primary measures of speed, but results were similar regardless of whether absolute or relative measure of speed were used. Endurance was quantified as the decrease in speed after a set duration of continuous speed trials.

Reconstructing emydid phylogeny

Stephens & Wiens (2003) provided a comprehensive phylogeny for emydid turtles based on combined molecular and morphological data. However, in that study, many species lacked molecular data, many parts of the tree were weakly supported (by parsimony bootstrapping), and the combined data were not analysed using model-based methods. In order to provide an improved framework for our phylogenetic analyses of locomotor performance, we revisit emydid phylogeny for the 16 species included in this study using new data and methods.

A more detailed description of the methods used to reconstruct emydid phylogeny is given in the Appendix S1. Here we only briefly outline these methods. Emydid relationships were investigated using 4214 characters (930 parsimony-informative), which included new DNA sequences generated for this study, morphological data from previous work by the authors, and molecular and morphological data from the literature. Analyses presented here included the 16 species measured during performance trials and outgroups consisting of three species of Geoemydidae (*Mauremys caspica*, *Mauremys reevesi* and *Morenia petersi*), the sister group to Emydidae (Shaffer *et al.*, 1997; Krenz *et al.*, 2005). The analyses included morphological data to increase the sample size of independent characters and more accurately estimate phylogeny. Morphological data ($n = 245$ parsimony informative characters) were based on our

previously published data and 12 characters from the literature (Stephens & Wiens, 2003). Mitochondrial sequences from four gene regions were included. Published ND4 (NADH dehydrogenase subunit 4; 905 bp) and cytochrome *b* (1198 bp) sequences (Lamb *et al.*, 1994; Feldman & Parham, 2002; Spinks *et al.*, 2004) were supplemented with new sequences. Published mitochondrial ribosomal large subunit (16S hereafter; 559 bp) sequences (Bickham *et al.*, 1996) and control region (345 bp) sequences (Lamb *et al.*, 1994) were also included. Nuclear sequence data [R35 (RNA fingerprint protein 35) nuclear intron; 948 bp] were new to this study. See Appendix S1 for a table of sequences used in the study, GenBank accession numbers, and a list of primers used for amplification and sequencing.

Data were analysed using parsimony (with PAUP* 4.0b10 Swofford, 2002) and Bayesian methods (MRBAYES version 3.0b4, Huelsenbeck & Ronquist, 2001). Prior to analysis of the combined data, we used the method of Wiens (1998) to assess incongruence between data sets. We favour this approach over global tests of incongruence because we were interested in the extent, location and causes of strongly supported incongruence, rather than the simplistic question of whether there is any incongruence between the datasets at all. Each data set (morphological data and the five gene regions) was initially analysed alone to look for areas of incongruence (i.e. conflicting hypotheses of relationships) that are strongly supported by two or more data sets, based on parsimony bootstrap values and Bayesian posterior probabilities (Wiens, 1998). Strong support was defined as bootstrap values $\geq 70\%$ (Hillis & Bull, 1993; but see also their extensive caveats) and Bayesian posterior probabilities (Pp) ≥ 0.95 (see Wilcox *et al.*, 2002; Alfaro *et al.*, 2003; Erixon *et al.*, 2003). No strongly supported incongruence between genes or between molecular data and morphology was found, and all data sets were therefore combined (but treated as distinct partitions in the Bayesian analysis), in order to increase the overall sample size of characters. Results from parsimony and Bayesian methods were largely congruent (see below).

The inclusion of non-molecular characters when estimating a tree that is then used to analyse phenotypic data is somewhat contentious (e.g. Coddington, 1988; Armbruster, 1993; de Queiroz, 1996; Luckow & Bruneau, 1997). To verify that the results of the analyses presented were not biased by the inclusion of morphological data during phylogeny estimation or the estimation of branch lengths (see below) all comparative analyses were repeated using a tree estimated solely from molecular data and using branch lengths estimated both enforcing and relaxing a molecular clock assumption. Comparative analyses were also repeated on a tree estimated by combining data from this study with data from all emydid taxa (from Stephens & Wiens, 2003) to ensure that the results were not biased by the exclusion of taxa during phylogeny estimation. The results of both sets of

additional comparative analyses were identical to those presented here with respect to direction of correlations and statistical significance (see Appendix S2).

Comparative analysis of locomotor data

We tested for evidence of trade-offs using simple linear regression of raw species performance data, independent contrasts, and reconstructed changes in performance (minimum evolution method of Martins & Garland, 1991). Aquatic speed was compared to terrestrial speed, aquatic speed was compared to aquatic endurance and terrestrial speed to terrestrial endurance. Given that it was difficult to obtain measurements of both aquatic endurance and terrestrial endurance for every species, we also compared overall speed and overall endurance. To compare overall speed to overall endurance, the average of aquatic and terrestrial speed observed for each species was compared to whichever endurance (aquatic or terrestrial) was recorded for each species (the average of both aquatic and terrestrial endurance was used for the species for which both could be measured). Phylogenetically independent contrasts (Felsenstein, 1985) for each variable were calculated using either estimated branch lengths or setting all branch lengths to a value of one. Branch lengths were estimated primarily based on the average length of each branch from the pooled post-burn-in trees from the Bayesian analysis of the combined data. However, additional analyses were also performed using branch lengths from the molecular data alone, both with and without a molecular clock. Independent contrasts were calculated using COMPARE version 4.6b (Martins, 2004). Regression analysis of contrasts used a model with no intercept (i.e. fitting regression lines through the origin; Garland *et al.*, 1992).

To determine if ancestral trait reconstructions supported the hypothesis of evolutionary trade-offs in performance, the amount of change in a given performance variable on a given branch was estimated as the difference in the values reconstructed for the ancestral and descendant node (for internal branches) and between the extant species and its most recent ancestral node (for terminal branches). Ancestral trait values were reconstructed using linear generalized least squares (Martins & Hansen, 1997) implemented in COMPARE v. 4.6b (Martins, 2004), primarily using branch lengths estimated from the Bayesian analysis of the combined data (as above). Changes in one trait were then regressed against changes in another trait, forcing the regression through the origin. Evolutionary trade-offs would be supported if reconstructed increases in performance in one trait are associated with reconstructed decreases in another trait on the same branch of the phylogeny. The statistical significance of the correlation between reconstructed changes was calculated in MICROSOFT EXCEL™ version 11.2.5 for Macintosh (Microsoft Corporation, Redmond, Washington, USA) using a *t*-test for the

statistical significance of *r* (as described in Sokal & Rohlf, 1995) with $N - 2$ d.f. where N was the number of species included in the analysis (following Martins & Garland, 1991; Pagel, 1993). This method gives identical results to looking up *r* in a table of critical values (e.g. Rohlf & Sokal, 1995). All regression analyses were performed using STATVIEW™ version 4.51 for Macintosh (Abacus Concepts Inc., Piscataway, New Jersey, USA).

Results were qualitatively identical in all comparisons of speed and endurance, regardless of whether aquatic speed and endurance or overall (averaged aquatic and terrestrial) speed and endurance were compared, and regardless of whether absolute (cm s^{-1}) or relative (carapace lengths/ second) measures of speed were used. The trend and strength of correlations were similar in comparisons of terrestrial speed and endurance, however these comparisons were not statistically significant due to the limited number of species that could be included (terrestrial endurance could be measured in only five species). Note also that there was no relationship between body size (carapace length) and endurance in our data ($r = -0.089$, $P = 0.750$ in regression analysis). For the sake of brevity, only comparisons of overall endurance and relative speed are presented.

Finally, we used both conventional ANOVA and phylogenetic generalized-least-squares (PGLS) ANOVA (Martins & Hansen, 1997; Ord & Martins, 2006) to test if variation in these locomotor variables was related to ecological diversification in habitat usage in emydids (i.e. we tested for an association between values of speed and endurance and each habitat type). For these analyses, we grouped emydids into three different categories of habitat use (Table 1; based on Ernst & Barbour, 1989; Ernst *et al.*, 1994). We considered 'aquatic' species to be those that feed or forage only in water. 'Terrestrial' species are those that feed only on land, and that rarely enter water save to thermoregulate or cross between terrestrial habitats. 'Semi-terrestrial' species are those reported to forage in both aquatic and terrestrial habitats in the wild. Note that these categories emphasize foraging patterns more strongly than habitat categories used in the past (e.g. Stephens & Wiens, 2003), but can be defined more objectively. There is also some disagreement over whether *Emydoidea blandingii* feeds terrestrially in the wild (reviewed in Ernst *et al.*, 1994). We therefore repeated analyses scoring this species both as aquatic and as semi-terrestrial. Both sets of analyses had qualitatively identical results (with respect to statistical significance), and only the results of analyses where *Emydoidea* is scored as semi-terrestrial are reported.

We first performed conventional ANOVA (implemented in JMP™ version 3.2.1). We then performed PGLS ANOVA (Martins & Hansen, 1997) on the same variables following the procedure outlined in Ord & Martins (2006). This alternative to phylogenetic ANOVA (Garland *et al.*, 1993) has the slight advantage of directly estimating a coefficient of determination for a continuous and discrete

variable after phylogenetic autocorrelation in both is accounted for. Two dichotomous dummy variables were used to represent the three habitat categories (i.e. $n - 1$ where n is the number of categories to be represented, following Bowerman & O'Connell, 1990; Ord & Martins, 2006), and these were regressed against speed and endurance using the PGLS method implemented in COMPARE. Ord & Martins (2006) describe coefficients of determination from PGLS ANOVA as roughly equivalent to Pearson product-moment correlation coefficients from conventional bivariate regression analyses. We therefore assessed the statistical significance of observed correlations using the t -test for the statistical significance of r described above. The tree topology and branch lengths for these analyses were estimated from the Bayesian analysis of the combined data (i.e. Fig. 1). PGLS ANOVA were repeated using both estimated and equal branch lengths.

We also reconstructed the evolution of habitat use on the tree, although these reconstructions were not used in any of the statistical hypothesis tests of locomotor performance. Reconstructions were performed on an expanded set of species beyond the 16 used in analyses of locomotor performance to ensure that the results were not biased by incomplete taxon sampling. Here (i.e. in Fig. 1) we only depict results for the 16 species included in this study. The results of the complete reconstruction

are available in Appendix S3. We used both parsimony and likelihood reconstruction methods implemented in MESQUITE v. 1.12 (Maddison & Maddison, 2006). Parsimony reconstructions were performed both treating habitat use as unordered, and as ordered (with semi-terrestrial habitat use as intermediate between terrestrial and aquatic). Maximum likelihood reconstructions used the Mk1 model of character evolution (Lewis, 2001), with a single estimated rate (in this case 2.294 per unit branch length) for all transitions between states. The statistical significance of the reconstructed state at each node was evaluated using a likelihood ratio test, where the likelihood of the character state with the highest likelihood was compared with that of the state with the second highest likelihood (Schluter *et al.*, 1997).

Results

Relationships among the 16 species generally were strongly supported by both parsimony and Bayesian analyses (Fig. 1) and are similar to those postulated by Stephens & Wiens (2003) save for the position of *Clemmys guttata*. The tree estimated for the 16 species of this study was identical regardless of whether the morphological data were included or excluded during phylogeny estimation. When additional taxa are included, the results were again similar with respect to the 16 species included

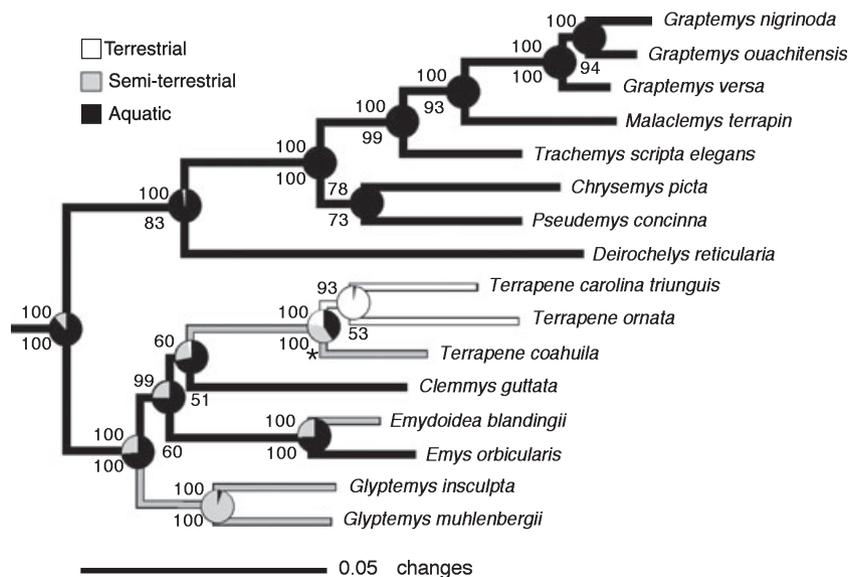


Fig. 1 Phylogeny and evolution of habitat use in emydid turtles. Phylogeny is based on a partitioned Bayesian analysis of the combined morphological and molecular data. Numbers above each branch indicate Bayesian posterior probabilities ($\times 100$), whereas numbers below each branch indicate bootstrap support (%) from a parsimony analysis of the combined data (bootstrap percentages ≤ 50 are not reported). Branch lengths represent average lengths from among the post-burn-in trees. Outgroup taxa are not shown. Shading on branches indicates ancestral reconstructions of habitat as an ordered character using parsimony. Pie charts indicate maximum likelihood reconstructions, where the size of each wedge and shading represents the relative likelihood of each of the three character states. For all but one branch (indicated by *), the character state with the highest likelihood had significantly greater support than the other two character states (based on a likelihood ratio test). Reconstructions shown are from an analysis which incorporated additional species to the 16 species of this study (shown in Fig. S1 in Appendix S3).

in this study, save that (a) *Clemmys guttata* appears as the sister to the *Emys-Emydoidea* clade rather than *Terrapene*, (b) *Terrapene coahuila* appears as the sister to *T. carolina* (rather than *T. ornata*) and (c) *G. versa* appears as the sister to *G. nigrinoda* (rather than *G. ouachitensis*). This alternate tree is shown in Fig. S1 in Appendix S2. No incongruence between these analyses was strongly supported in both. Thus the placement of *G. nigrinoda* is strongly supported in Fig. 1 but not the analysis including additional species, whereas the position of *C. guttata* and the sister of *T. carolina* are strongly supported (by bootstrapping) in the latter analysis and weakly supported in Fig. 1. The results of all comparative analyses reported below are identical, with respect to direction of correlation and statistical significance, regardless of which tree topology or branch length estimates are used (see Appendix S2 for the results of these additional analyses). Reconstructions of ancestral states using both parsimony and likelihood suggest that the ancestor of emydids was aquatic, and that there were several transitions between semi-terrestrial and aquatic habitat use and one transition to terrestriality. If habitat is treated as unordered during parsimony reconstructions, the results are identical to those shown in Fig. 1 save that the character state of the ancestor of *Terrapene* is ambiguous, with semi-terrestrial and terrestrial being equally parsimonious. For likelihood reconstructions, the character state at each node with the highest likelihood had significantly greater support than the other two character states (based on a likelihood ratio test) for all but one branch.

Raw values of aquatic and terrestrial speed (Table 1) were positively correlated (Fig. 2a; $r = 0.642$, $P = 0.013$), rather than negatively correlated, as would be expected for variables involved in an ecological trade-off. However, there was no significant relationship between phylogenetically independent contrasts of relative aquatic and terrestrial speed using either estimated ($r = 0.293$, $P = 0.331$) or equal branch lengths ($r = 0.257$, $P = 0.395$), nor between reconstructed changes in relative aquatic and terrestrial speed using estimated (Fig. 3a; $r = 0.297$, $P = 0.775$) or equal branch lengths ($r = 0.354$, $P = 0.684$). Overall, these results indicate that there is no trade-off in speed between aquatic and terrestrial environments in emydids.

Relative speed and endurance were negatively correlated in analyses of the raw data (Fig. 2b; $r = -0.815$, $P < 0.001$) and independent contrasts using both estimated ($r = -0.504$, $P = 0.038$) and equal branch lengths ($r = -0.738$, $P = 0.017$). Reconstructed changes in endurance were also negatively correlated with changes in speed using estimated (Fig. 3b; $r = -0.700$, $P = 0.036$) and equal branch lengths ($r = -0.699$, $P = 0.037$). These results indicate an evolutionary trade-off between speed and endurance.

Habitat use was correlated with differences in speed ($F = 11.06$, $P = 0.002$) and endurance ($F = 5.567$,

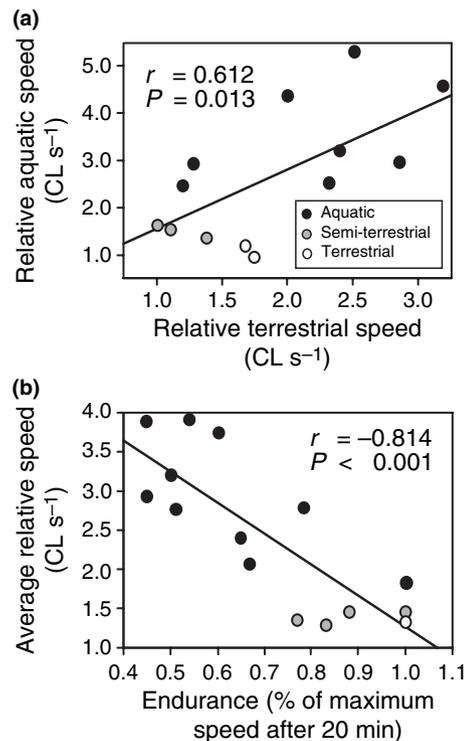


Fig. 2 Locomotor performance and ecology of emydid species, showing relationships between raw values for: (a) aquatic and terrestrial speed, (b) speed and endurance (legend for habitat shown in 2a).

$P = 0.020$) between species using standard ANOVA. Speed was correlated with habitat use in PGLS ANOVA using both estimated ($r = 0.656$, $P = 0.006$) and equal ($r = 0.658$, $P = 0.006$) branch lengths. Endurance was also correlated with differences in habitat use using both estimated ($r = 0.545$, $P = 0.035$) and equal ($r = 0.661$, $P = 0.007$) branch lengths. Aquatic species generally had low endurance and high speed, whereas semi-terrestrial and terrestrial species had high endurance and low speed (Fig. 2).

Discussion

Evolutionary trade-offs and ecological diversification

In general, organisms are thought to ecologically specialize and diversify in habitat use because increased performance in one environment may come at a cost to performance in another environment (MacArthur, 1972; Futuyma & Moreno, 1988; Pianka, 2000). Contrary to these expectations, we found no evidence of an evolutionary trade-off in aquatic and terrestrial speed in emydids. Instead, specialization for locomotion in different habitats has seemingly involved trade-offs in speed vs. endurance, not changes in speed or endurance in

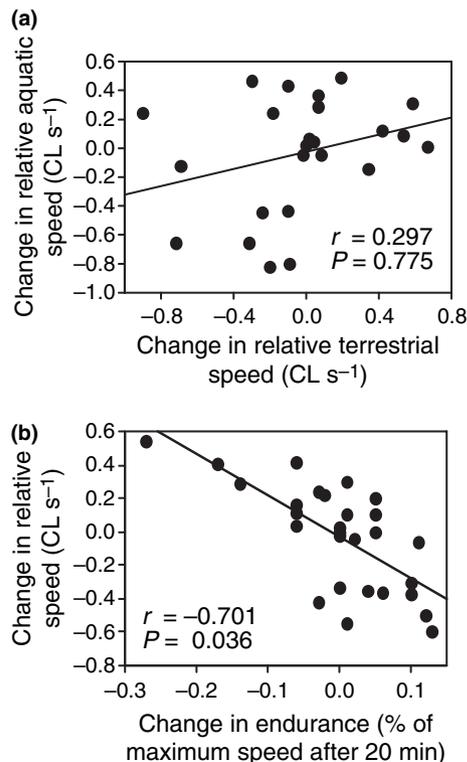


Fig. 3 Regression analyses of (a) reconstructed changes in relative aquatic speed on each branch vs. reconstructed changes in relative terrestrial speed and (b) reconstructed changes in relative speed (average of relative aquatic and terrestrial speed) on each branch vs. reconstructed changes in endurance using branch lengths estimated from the Bayesian analysis of the combined data.

different environments (Fig. 2). Specifically, aquatic species tend to have higher speed in both aquatic and terrestrial environments, whereas terrestrial and semi-terrestrial species tend to have lower speed but greater endurance in both environments. To date, only one other phylogenetic comparative study has directly tested for trade-offs between aquatic and terrestrial locomotion (Gvozdik & van Damme, 2006). That study also showed aquatic and terrestrial speed to be positively correlated in semi-terrestrial newts (i.e. they found no evidence of a trade-off). However, that study did not compare fully aquatic and terrestrial species or consider endurance.

One emydid species differed from the general pattern of variation in speed, endurance and habitat use. *Deirochelys reticularia* showed much lower speed and higher endurance than other aquatic species (Table 1). This species and the semi-terrestrial species *Emydoidea blandingii* are both thought to be specialized for pharyngeal feeding, in which prey are captured by rapidly thrusting out the head and neck while expanding the buccopharyngeal cavity (Ernst *et al.*, 1994). Previous studies have shown that *E. blandingii* and *D. reticularia*

exhibit a remarkable degree of convergence in skeletal morphology (e.g. Loveridge & Williams, 1957; McDowell, 1964), which is generally assumed to be related to pharyngeal feeding (Bramble, 1974; Ernst *et al.*, 1994; Stephens & Wiens, 2003). We speculate that this convergence in feeding mode may also be related to convergence in the locomotor performance of *Deirochelys* with *Emydoidea*, but further studies are needed to determine the relationships between emydid skeletal morphology, foraging modes and locomotor performance.

A negative correlation between speed and endurance is generally thought to be a fundamental trade-off in locomotor performance that occurs virtually in all animals because of the physiology of skeletal muscle and the biomechanics of skeletomuscular systems (e.g. Rome *et al.*, 1988; Rome, 2002; Biewener, 2003). Surprisingly, the evidence for this trade-off at the level of whole organism performance has been somewhat equivocal. Although there have been numerous intraspecific studies, they have often shown no correlation (e.g. Perry *et al.*, 2004) or positive correlations (e.g. Garland, 1988; Secor *et al.*, 1992; Pinch & Claussen, 2003) between the speed and endurance of individuals (reviewed in Vanhooydonck *et al.*, 2001). In contrast, only two previous studies (Huey *et al.*, 1984; Vanhooydonck *et al.*, 2001) have directly tested for a trade-off between speed and endurance among species, and both supported it.

Our results also suggest that an evolutionary trade-off between speed and endurance may underlie ecological specialization to different habitats. Previous comparative studies showing speed and endurance trade-offs (Huey *et al.*, 1984; Vanhooydonck *et al.*, 2001) implicitly assumed that the trade-offs they observed were related to differences in habitat use, but did not test for this relationship. Given that interspecific trade-offs between speed and endurance may be particularly widespread, this hypothesis should be tested in other ecologically diverse groups of organisms.

Why might the speed-endurance trade-off be important for habitat specialization in emydids? All emydids are active foragers, however aquatic species often spend more time basking than semi-terrestrial and terrestrial species (Ernst *et al.*, 1994). In addition, the heavy shell of turtles may make rapid flight difficult in terrestrial environments but may require considerable stamina for normal movement. The only strictly terrestrial emydids (*Terrapene*) have evolved a special defensive mechanism (hinged, closeable shell) that does not involve fleeing (Bramble, 1974). In contrast, many aquatic emydids spend much of their time basking motionlessly adjacent to water, and may quickly slide into the water and swim rapidly away when disturbed, in an aquatic environment where the weight of the shell need not be supported (Ernst *et al.*, 1994). Aquatic species may have little need for endurance, compared with more terrestrial species that frequently have to bear the full weight of their shell.

The present study may be the first to directly compare the speed and endurance of terrestrial and aquatic species. Given that terrestrial species may typically have to 'carry their weight', as opposed to many aquatic species that are neutrally buoyant, we speculate that trade-offs between speed and endurance might be a common feature of evolutionary transitions between aquatic and terrestrial habitats in tetrapods and other organisms.

Documenting evolutionary trade-offs

In this study, we propose an explicit definition of evolutionary trade-offs and apply this criterion in emydid turtles. Many comparative studies have attempted to document evolutionary trade-offs between traits among species (e.g. Irschick, 2002; Jakobsson & Eriksson, 2003; Poulin & Mouillot, 2004; Yurewicz, 2004), but only some have taken phylogeny into account. Regression analyses of raw species data clearly are useful to look for trade-offs in the broad sense of 'any negative correlation between traits'. However, a non-phylogenetic approach may be problematic when looking for evolutionary trade-offs among species because it does not directly address evolutionary increases or decreases in performance. In fact, given two traits among a set of species, a negative correlation between traits among species could arise if the values of the two traits simply increased in different lineages, without any evolutionary decrease in performance in either trait (i.e. trait A increases in clade 1 and trait B increases in clade 2). In other words, a non-phylogenetic approach may suggest an evolutionary trade-off when none has occurred, but not for the same reason that typically makes non-phylogenetic methods problematic (i.e. the non-independence of species data, Felsenstein, 1985).

Studies of experimental evolution can show directly whether trade-offs currently occur among traits of individuals in a species or population (i.e. a microevolutionary trade-off), and what developmental or genetic constraints might account for such trade-offs (reviewed by Brakefield, 2006; Roff & Fairbairn, 2007). However, such studies do not address whether trade-offs explain patterns of diversity among species (i.e. a macroevolutionary trade-off). Thus, a comparative approach is essential to understanding these broader evolutionary patterns (e.g. Irschick, 2002; Poulin & Mouillot, 2004; Brakefield & Roskam, 2006).

The most direct way of addressing evolutionary trade-offs among species is by inferring evolutionary increases and decreases in performance or fitness on a phylogeny using ancestral trait reconstruction (minimum evolution method), as we have done here. So far, minimum evolution has rarely been applied to look for evolutionary trade-offs, and the one study (Moran, 2004) that did not include an explicit definition of an evolutionary trade-off. Another approach is to use independent contrasts of raw trait values to correct for the phylogenetic

relatedness of species (e.g. Jakobsson & Eriksson, 2000; Irschick, 2002; Poulin & Mouillot, 2004). This latter approach does not directly address evolutionary increases and decreases in performance either, but it seems likely to give similar results to the minimum evolution approach under many circumstances. For example, in our study both methods yielded identical results with respect to the direction and statistical significance of correlations among variables. Simulation studies (Martins & Garland, 1991) suggest that the minimum evolution method can estimate the correlation between evolutionary changes in two traits more accurately than independent contrasts under many conditions. Unfortunately, they also showed that under some extreme branch length conditions the minimum evolution method has inflated rates of Type I error, and both methods may fail when the data grossly violate the Brownian motion model of character evolution. Researchers interested in the evolution of ecological trade-offs should keep in mind what evolutionary pattern they expect under this hypothesis, and whether the methods that they use can directly test those predictions.

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References

- Alfaro, M.E., Zoller, S. & Lutzoni, F. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in

- assessing phylogenetic confidence *Mol. Biol. Evol.* **20**: 255–266.
- Arendt, J.D. 2003. Reduced burst speed is a cost of rapid growth in anuran tadpoles: problems of autocorrelation and inferences about growth rates. *Funct. Ecol.* **17**: 328–334.
- Armbruster, W.S. 1993. Evolution of plant pollination systems: hypotheses and tests with the neotropical vine *Dalechampia*. *Evolution* **47**: 1480–1505.
- Badyaev, A.V. 2002. Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. *Evolution* **56**: 412–419.
- Bickham, J.W., Lamb, T., Minx, P. & Patton, J.C. 1996. Molecular systematics of the genus *Clemmys* and the intergeneric relationships of emydid turtles. *Herpetologica* **52**: 89–97.
- Biewener, A.A. 2003. *Animal Locomotion*. Oxford University Press, Oxford, England.
- Bowerman, B.L. & O'Connell, R.T. 1990. *Linear Statistical Models, an Applied Approach*. Duxbury Press, Belmont, California.
- Brakefield, P.M. 2006. Evo-devo and constraints on selection. *Trends Ecol. Evol.* **21**: 362–368.
- Brakefield, P.M. & Roskam, J.C. 2006. Exploring evolutionary constraints is a task for an integrative evolutionary biology. *Am. Nat.* **168**: S4–S13.
- Bramble, D.M. 1974. Emydid shell kinesis: biomechanics and evolution. *Copeia* **1974**: 102–109.
- Coddington, J. 1988. Cladistic tests of adaptational hypotheses. *Cladistics* **9**: 305–342.
- Erixon, P., Svennblad, B., Britton, T. & Oxelman, B. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Syst. Biol.* **52**: 665–673.
- Ernst, C.H. & Barbour, R.W. 1989. *Turtles of the World*. Smithsonian Institution Press, Washington, DC.
- Ernst, C.H., Lovich, J.E. & Barbour, R.W. 1994. *Turtles of the United States and Canada*. Smithsonian Institution Press, Washington, DC.
- Feldman, C.R. & Parham, J.F. 2002. Molecular phylogenetics of emydid turtles: taxonomic revision and the evolution of shell kinesis. *Mol. Phylogenet. Evol.* **22**: 388–398.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.
- Fry, J.D. 1996. The evolution of host specialization: are trade-offs overrated? *Am. Nat.* **148**: S84–S107.
- Futuyma, D.J. 2005. *Evolution*. Sinauer Associates, Sunderland, MD.
- Futuyma, D.J. & Moreno, G. 1988. The evolution of ecological specialization. *Ann. Rev. Ecol. Syst.* **19**: 207–233.
- Garland, T. Jr 1988. Genetic basis of activity metabolism. I. Inheritance of speed, stamina, and antipredator displays in the garter snake *Thamnophis sirtalis*. *Evolution* **42**: 335–350.
- Garland, T. Jr, Dickerman, A.W., Janis, C.M. & Jones, J.A. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* **42**: 265–292.
- Garland, T. Jr, Harvey, P.H. & Ives, A.R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**: 18–32.
- Gillis, G.B. & Blob, R.W. 2001. How muscles accommodate movement in different environments: aquatic vs. terrestrial locomotion in vertebrates. *Comp. Biochem. Physiol. A* **131**: 61–75.
- Gvozdk, L. & van Damme, R. 2006. *Triturus* newts defy the running-swimming dilemma. *Evolution* **60**: 2110–2121.
- Hillis, D.M. & Bull, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* **42**: 182–192.
- Huelsenbeck, J.P. & Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.
- Huey, R.B., Bennett, A.F., John-Alder, H. & Nagy, K.A. 1984. Locomotor capacity and foraging behavior of Kalahari lacertid lizards. *Anim. Behav.* **32**: 41–50.
- Irschick, D.J. 2002. Evolutionary approaches for studying functional morphology: examples from studies of performance capacities. *Integr. Comp. Bio.* **42**: 278–290.
- Irschick, D.J., Herrel, A., Vanhooydonck, B., Huyghe, K. & Van Damme, R. 2005. Locomotor compensation creates a mismatch between laboratory and field estimates of escape speed in lizards: a cautionary tale for performance-to-fitness studies. *Evolution* **59**: 1579–1587.
- Jakobsson, A. & Eriksson, O. 2000. A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos* **88**: 494–502.
- Jakobsson, A. & Eriksson, O. 2003. Trade-offs between dispersal and competitive ability: a comparative study of wind-dispersed Asteraceae forbs. *Evol. Ecol.* **17**: 233–246.
- Krenz, J.G., Naylor, G.J.P., Shaffer, H.B. & Janzen, F.J. 2005. Molecular phylogenetics and evolution of turtles. *Mol. Phylogenet. Evol.* **37**: 178–191.
- Lamb, T., Lydeard, C., Walker, R. & Gibbons, J.W. 1994. Molecular systematics of the map turtles (*Graptemys*): a comparison of mitochondrial restriction site versus sequence data. *Syst. Biol.* **43**: 543–599.
- Lewis, P.O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* **50**: 913–925.
- Losos, J.B. & Miles, D.B. 1994. Adaptation, constraint, and the comparative method: Phylogenetic issues and methods. In: *Ecological Morphology* (P. C. Wainwright & S. Reilly, eds), pp. 60–98. University of Chicago Press, Chicago.
- Loveridge, A. & Williams, E.E. 1957. Revision of the African tortoises and turtles of the suborder Cryptodira. *Bull. Mus. Comp. Zool.* **115**: 163–557.
- Luckow, M. & Bruneau, A. 1997. Circularity and independence in phylogenetic tests of ecological hypotheses. *Cladistics* **13**: 145–151.
- MacArthur, R.H. 1972. *Geographical Ecology*. Harper and Row, New York.
- Maddison, W.P. & Maddison, D.R. 2006. *Mesquite: A Modular System for Evolutionary Analysis*. Version 1.12 <http://mesquiteproject.org>.
- Martins, E.P. 2004. COMPARE, version 4.6b. Computer Programs for the Statistical Analysis of Comparative Data. Distributed by the author at <http://www.indiana.edu/~martinsl/compare/>. Department of Biology, Indiana University, Bloomington, Indiana.
- Martins, E.P. & Garland, T. Jr 1991. Phylogenetic analyses and the correlated evolution of continuous characters: a simulation study. *Evolution* **45**: 534–557.
- Martins, E.P. & Hansen, T.F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into analysis of interspecific data. *Am. Nat.* **149**: 646–667.
- McDowell, S.B. 1964. Partition of the genus *Clemmys* and related problems in the taxonomy of the aquatic Testudinidae. *Proc. Zool. Soc. Lond.* **143**: 239–279.

- Miner, B.G. 2005. Evolution of feeding structure plasticity in marine invertebrate larvae: a possible trade-off between arm length and stomach size. *J. Exp. Marine Bio. Ecol.* **315**: 117–125.
- Moran, A.L. 2004. Egg size evolution in tropical American arcid bivalves: the comparative method and the fossil record. *Evolution* **58**: 2718–2733.
- Ord, T.J. & Martins, E.P. 2006. Tracing the origins of signal diversity in anole lizards: phylogenetic approaches to inferring the evolution of complex behavior. *Anim. Behav.* **71**: 1411–1429.
- Pagel, M.D. 1993. Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. *J. Theor. Bio.* **164**: 191–205.
- Perry, G., Levering, K., Girard, I. & Garland, T. Jr 2004. Locomotor performance and social dominance in male *Anolis cristatellus*. *Anim. Behav.* **67**: 37–47.
- Pianka, E.R. 2000. *Evolutionary Ecology*, 6th edn. Addison Wesley Longman, New York.
- Pinch, F.C. & Claussen, D.L. 2003. Effects of temperature and slope on the sprint speed and stamina of the eastern fence lizard, *Sceloporus undulatus*. *J. Herpetol.* **37**: 671–679.
- Poulin, R. & Mouillot, D. 2004. The relationship between specialization and local abundance: the case of helminth parasites of birds. *Oecologia* **140**: 372–378.
- de Queiroz, K. 1996. Including the characters of interest during tree reconstruction and the problems of circularity and bias in studies of character evolution. *Am. Nat.* **148**: 700–708.
- Roff, D.A. & Fairbairn, D.J. 2007. The evolution of trade-offs: where are we? *J. Evol. Biol.* **20**: 433–447.
- Rohlf, F.J. & Sokal, R.R. 1995. *Statistical Tables*. W. H. Freeman and Company, New York, NY.
- Rome, L. 2002. The design of vertebrate muscular systems: comparative and integrative approaches. *Clin. Orthop.* **403S**: S59–S76.
- Rome, L.C., Funke, R.P., McNeil Alexander, R., Lutz, G., Aldridge, H., Scott, F. & Freadman, M. 1988. Why animals have different muscle fiber types. *Nature* **335**: 824–827.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford, England.
- Schluter, D., Price, T., Mooers, A.Ø. & Ludwig, D. 1997. Likelihood of ancestor states in adaptive radiation. *Evolution* **51**: 1699–1711.
- Secor, S.M., Jayne, B.C. & Bennett, A.F. 1992. Locomotor performance and energetic cost of sidewinding by the snake *Crotalus cerastes*. *J. Exp. Bio.* **163**: 1–14.
- Shaffer, H.B., Meylan, P. & McKnight, M.L. 1997. Tests of turtle phylogeny: molecular, morphological, and paleontological approaches. *Syst. Biol.* **46**: 235–268.
- Shubin, N., Tabin, C. & Carroll, S. 1997. Fossils, genes, and the evolution of animal limbs. *Nature* **388**: 639–648.
- Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*, 3rd edn. W. H. Freeman and Company, New York, NY.
- Spinks, P.Q., Shaffer, H.B., Iverson, J.B. & McCord, W.P. 2004. Phylogenetic hypotheses for the turtle family Geoemydidae. *Mol. Phylogenet. Evol.* **32**: 164–182.
- Stephens, P.R. & Wiens, J.J. 2003. Ecological diversification and phylogeny of emydid turtles. *Biol. J. Linn. Soc.* **79**: 577–610.
- Swofford, D.L. 2002. *PAUP**: Phylogenetic analysis using parsimony*, v. 4.0b10. Sinauer Associates, Sunderland, MD.
- Van Ballegooijen, W.M. & Boerlijst, M.C. 2004. Emergent trade-offs and selection for outbreak frequency in spatial epidemics. *Proc. Natl Acad. Sci. U.S.A.* **52**: 18246–18250.
- Vanhooydonck, B., Van Damme, R. & Aerts, P. 2001. Speed and stamina trade-off in lacertid lizards. *Evolution* **55**: 1040–1048.
- Wiens, J.J. 1998. Combining data sets with different phylogenetic histories. *Syst. Biol.* **47**: 568–581.
- Wilcox, T.P., Zwickl, D.J., Heath, T.A. & Hillis, D.M. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Mol. Phylogenet. Evol.* **25**: 361–371.
- Yoshida, T., Hairston, N.G. Jr & Ellner, S.P. 2004. Evolutionary trade-off between defense against grazing and competitive ability in unicellular alga, *Chlorella vulgaris*. *Proc. R. Soc. Lond.* **271**: 1947–1953.
- Yurewicz, K.L. 2004. A growth/mortality trade-off in larval salamanders and the coexistence of intraguild predators and prey. *Oecologia* **138**: 102–111.
- Zug, G.R. 1971. Buoyancy, locomotion, morphology of the pelvic girdle and hindlimb, and systematics of cryptodiran turtles. *Misc. Publ. Mus. Zool. Univ. Mich.* **142**: 1–90.

Supplementary material

The following supplementary material is available for this article:

Appendix S1 Detailed materials and methods.

Appendix S2 Testing the robustness of the phylogenetic results and comparative analyses.

Appendix S3 Reconstructing patterns of habitat use.

Table S1 Sources of sequences used in study.

Table S2 Oligonucleotide primers used for DNA amplification and sequencing.

Table S3 Phylogenetic comparisons of performance variables.

Table S4 Phylogenetic analyses of the effects of habitat on performance variables.

Figure S1 Alternate tree topology used for phylogenetic comparative analyses of performance variables.

Figure S2 Evolution of habitat use in emydid turtles.

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